

CLAIMS

1. Enzymatically-active protein comprising:

- a GFAT sequence and at least one purification tag sequence, the purification tag sequence being inserted between two consecutive amino acids of the GFAT sequence, or
- a sequence deriving from the preceding sequence by suppression, insertion or mutation of at least one amino acid, provided that said protein has an enzyme activity, or
- a sequence having at least 35% sequence identity and/or at least 44% sequence similarity with one of the preceding sequences, provided that said protein has an enzyme activity.

2. Protein according to claim 1, in which the GFAT sequence corresponds to a bacterial or eukaryotic, in particular plant, fungal or animal, in particular insect or mammal, more particularly human GFAT sequence.

3. Protein according to claim 1 or 2, in which the purification tag sequence is inserted between two consecutive amino acids of the GFAT sequence, said amino acids being included in:

- a part of the GFAT sequence corresponding and/or being homologous to the sequence extending approximately between amino acids 30 to 80 of the *Escherichia coli* GFAT, or
- a part of the GFAT sequence corresponding and/or being homologous to the sequence extending approximately between amino acids 220 to 230 of the *Escherichia coli* GFAT, or
- a part of the GFAT sequence corresponding and/or being homologous to the sequence extending approximately between amino acids 235 to 250 of the *Escherichia coli* GFAT.

4. Protein according to one of claims 1 to 3, in which the purification tag sequence is inserted between two consecutive amino acids of a human GFAT sequence, said amino acids being included between amino acids 40 to 50, 290 to 330, and/or 340 to 370 of said human GFAT sequence.

5 5. Protein according to one of claims 1 to 4, in which the GFAT sequence corresponds to:

- SEQ ID NO: 2, corresponding to the sequence of the human GFAT1,
- SEQ ID NO: 4, corresponding to the human GFAT2 sequence,
- SEQ ID NO: 6, corresponding to the human GFAT1Alt sequence.

10 6. Protein according to one of claims 1 to 5, in which the purification tag sequence is inserted between two consecutive amino acids, said amino acids being included between amino acids:

- 43 to 47, 298 to 306, and/or 342 to 347 of SEQ ID NO: 2
- 42 to 45, 299 to 307, and/or 343 to 348 of SEQ ID NO: 4
- 43 to 47, 316 to 324, and/or 360 to 365 of SEQ ID NO: 6

15 7. Protein according to claim 6, in which the purification tag sequence is inserted between amino acids:

- 299 and 300 of SEQ ID NO: 2.
- 300 and 301 of SEQ ID NO: 4
- 317 and 318 of SEQ ID NO: 6

20 8. Protein according to one of claims 1 to 7, in which the purification tag corresponds to a sequence of approximately 2 to approximately 10 amino acids, in particular approximately 4 to approximately 8 amino acids.

25 9. Protein according to one of claims 1 to 8, in which the purification tag is a hexa-histidine.

30 10. Protein according to claims 7 and 9 corresponding to the sequences:

- SEQ ID NO: 8, corresponding to the sequence SEQ ID NO: 2 in which a hexa-histidine is inserted between amino acids 299 and 300,
- SEQ ID NO: 10, corresponding to the sequence SEQ ID NO: 4 in which a hexa-histidine is inserted between amino acids 300 and 301, and
- SEQ ID NO: 12, corresponding to the sequence SEQ ID NO: 6 in which a hexa-histidine is inserted between amino acids 317 and 318.

11. Nucleic acid comprising or being constituted by a sequence coding for a protein according to one of claims 1 to 10.

5 12. Nucleic acid comprising or being constituted by the nucleotide sequence:

- SEQ ID NO: 7 coding for the protein SEQ ID NO: 8, or
- SEQ ID NO: 9 coding for the protein SEQ ID NO: 10, or
- SEQ ID NO: 11 coding for the protein SEQ ID NO: 12,

10 or by its complementary sequence, or being derived from said sequence by mutation, insertion or deletion of at least one nucleotide, provided that said nucleotide sequence codes for a enzymatically-active protein.

15 13. Eukaryotic or prokaryotic vector comprising a nucleic acid according to claim 11 or 12.

20 14. Purification process for a protein according to one of claims 1 to 10, from a solution comprising said protein, comprising a stage of bringing said solution into the presence of a compound binding specifically to the purification tag of said protein and a stage of separation of the complex formed by the binding of said protein to said compound from the other constituents of the solution.

25 15. Purification process according to claim 14, comprising a stage of bringing a solution comprising a protein according to claim 10 into the presence of a compound comprising a divalent metallic cation such as Ni^{2+} or Co^{2+} , in particular Ni^{2+} , and a stage of separation of the complex formed by the binding of the protein to said compound from the other constituents of the solution.

30 16. Purification process for a protein according to one of claims 1 to 10 in an enzymatically-active form, in particular at -80°C or at 4°C , comprising the addition of said protein to a solution comprising:

- approximately 1 mM to approximately 10 mM of fructose 6-phosphate, in particular approximately 1 mM,
- approximately 1 mM to approximately 5 mM of Tris(2-carboxyethyl)phosphine, in particular approximately 1 mM,

- approximately 5% to approximately 20% of glycerol, in particular approximately 10%.

5 **17.** Composition comprising an active GFAT protein, if appropriate bound to a purification tag, such as a protein according to one of claims 1 to 10, said protein being capable of being preserved in an enzymatically-active form, for at least 8 days at a temperature of 2°C to 10°C, in particular approximately 4°C, and for at least 12 months at a temperature of -100°C to -20°C, in particular approximately -80°C, said protein being in combination with:

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 - approximately 1 mM to approximately 10 mM of fructose 6-phosphate, in particular approximately 1 mM,
 - approximately 1 mM to approximately 5 mM of Tris(2-carboxyethyl)phosphine, in particular approximately 1 mM,
 - approximately 5% to approximately 20% of glycerol, in particular approximately 10%.
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18. Use of a protein according to one of claims 1 to 10, for the screening of compounds modifying the activity of said protein, in particular for the screening of said protein inhibitor.

20 **19.** Use according to claim 18, for the screening of compounds useful within the framework of the treatment or prevention of diabetes, in particular type II diabetes, obesity, acidosis, ketosis, arthritis, cancer, or osteoporosis.